SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials

FG-4497 was obtained from FibroGen, Inc. (San Francisco, CA, USA). For *in vivo* experiments, FG-4497 was dissolved in 5% dextrose, pH 8.5 to 9.2 (with sonication) at 10 mg/ml and dosed at 50 mg/kg. For cell culture experiments, FG-4497 was dissolved directly in cell culture media by sonication and used at 30 µM final concentration. Administration of 1-methyl-DL-tryptophan (1-MT; Sigma-Aldrich, St. Louis, MO, USA) was accomplished by addition the drinking water of treated mice at 5 mg/ml (Uyttenhove et al., 2003). L-689,560 (VWR, Radnor, PA, USA) was dosed according to published protocols (Ohtani et al., 2002). The JAK1/2 inhibitor INCB018424 (Ruxolitinib, Incyte, Alapocas, DE, USA) or vehicle (0.05% dimethylacetamide) were dosed at 90 mg/kg by oral gavage. Kynurenic-3,5,6,7,8-d_5 acid was purchased from CDN Isotopes, Pointe-Claire, Quebec, Canada. 1,2,3,4-$^{13}C_4$-alpha-ketoglutaric acid disodium salt and D4-succinic acid disodium salt were purchased from Cambridge Isotope Laboratories, Cambridge MA USA.

Uniformly labeled-[^{13}C] alpha-ketoglutarate was synthesized by Dr. H. H. Seltzman and colleagues at the Research Triangle Institute (RTI Study No. 0213994.002.009) through a contract with the NHLBI. Esterification of commercially available ^{13}C-U-alpha-ketoglutarate was carried out by azeotropic distillation of water. Briefly, 25g of ^{13}C-U-alpha-ketoglutarate was dissolved in 100ml methanol and 200ml chloroform and stirred with 0.5g toluene sulfonic acid. The solution was heated to 75°C and the distillate was collected and monitored by ^{13}CNMR. The compound was identified as esterified ^{13}C-alpha-ketoglutarate using GC-MS. The crude preparation was then subjected to stepwise chromatography, using ISCO silica gel columns, varied elution protocols and concentration, to achieve 95-98% purity.

Cell Culture

Mouse embryonic fibroblasts (MEFs) were obtained from *Egln1*+/+ and *Egln1-/-* littermates, and immortalized with a large T antigen K1 mutant in pLB(N)-CX (Clontech, Mountain View, CA, USA) modified for blasticidin selection. After selection for immortalized clones, cells were maintained in pyruvate-free DMEM containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin. Hepa-1c1c7 mouse hepatoma cells (ATCC, Manassas, VA, USA) were grown in MEM (Corning Mediatech, Manassas, VA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. 143B cybrid cells (Sullivan et al., 2015) were grown in DMEM supplemented with 1 mM sodium pyruvate and 0.1 mg/ml uridine. C2C12 myoblasts were maintained in DMEM containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S). Cells were differentiated into myotubes by changing media to DMEM with 2% horse serum containing 1% P/S for 5 days prior to experimentation. HL-1 cardiomyocytes were maintained in Claycomb medium containing 10% FBS, 1% P/S, 0.1 mM norepinephrine (Sigma), and 2 mM glutamine (Invitrogen, Carlsbad, CA). Egln1 WT and KO MEFs were maintained in DMEM containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S). For all metabolism experiments, studies were conducted in dialyzed FBS and pyruvate-free DMEM.

In Vitro Translation of Murine Egln1

A partial cDNA for murine Egln1 was cloned from mouse quadriceps RNA. A gene-specific RT primer (5'-GCTGTAGGTGACGTGGGTACT-3') was used for cDNA synthesis, followed by nested PCR (5'-CTAGAATTCATGGCCAGTGA-3'; 5'-GCTTAATTAACTAGACGTCTTTGCT3'). This amplicon was cloned between EcoR1 and Pac1 sites of pT7CFE1-NHIS-GST-CHA (Pierce Biotechnology, Rockford IL), sequenced, and found to be missing the 5' GC-rich region of Egln1. This region (1-377) was synthesized (Genewiz, South Plainfield, NJ) with addition of a 5'-Nde1 site. The partial Egln1 clone in pT7CFE1-NHIS-GST-CHA, and the synthesized gene segment, were digested with Nde1 and BamH1, and ligated to generate the complete gene. The final clone was sequenced, and demonstrated to be correct and in-frame with the 5' GST tag, with a stop codon prior to the N-terminal HA tag. Egln1 was translated using a 1-step Human High-Yield Mini IVT Kit (Pierce Biotechnology), separated by GST-column, and eluted by cleavage of the GST tag with HRV 3C protease (Pierce Biotechnology). Egln1 was quantified by polyacrylamide gel electrophoresis and silver staining, along with a standard curve of BSA.

Retroviral Expression

Flag-tagged, wild-type human EGLN1 expression vectors have been previously described (Lorenzo et al., 2014). The EGLN1 cDNA was subcloned into a pLenti construct with a CMV promoter using Gateway technology (Life Technologies). The H374R EGLN1 mutant (Ladroue et al., 2008) was generated by creation of a c.1121A→G mutation by site-directed mutagenesis.

A lentiviral construct containing three copies of the canonical hypoxia response elements (HRE) from *EPO* and a minimal thymidine kinase promoter upstream of firefly luciferase gene (pLenti-3xHRE-luciferase) was engineered by subcloning the SmaI and XbaI digest fragment of 3xHRE-TK-pGL3 (Yan et al., 2007) into a promoter-less pLenti vector created by digestion of pLenti6 with SmaI and XbaI.

Control pLKO.1 and Egln1 shRNA vectors (TRCN0000001043, TRCN0000009743, TRCN0000009739), all containing a Puromycin resistance cassette, were obtained from the Broad Institute TRC shRNA library.

Lentiviruses were produced by co-transfection (TransIT, Mirus Bio LLC, Madison, WI, USA) of 293TL cells with expression vectors along with the packaging constructs psPAX2 (GAG-pol) and pMD2.G (VSV-G). Viral supernatants were harvested at 48 hour and filtered through a 45 µm filter to remove cellular debris. Cells were infected by addition of viral supernatants and centrifugation (2250 rpm for 2 hours at 37°C) in the presence of 8 µg/ml of polybrene. For C2C12 studies, myoblasts were infected by addition of viral supernatants and centrifugation (2250 rpm for 2 hours at 37°C) in the presence of 8 µg/ml of polybrene. Puromycin (1 µg/ml) selection was begun at 24 hours after infection and continued through myotube differentiation period, with removal 24 hours prior to experimentation.

Luciferase Assay

Hepa-1c1c7 mouse hepatoma cells were infected with lentivirus containing HRE-luciferase. Hygromycin (200 µg/ml) selection was begun at 24 hours after infection and continued for 7 days until death of all uninfected control cells were dead. Cells were untreated, treated with FG-4497, or cultured in 1% hypoxia for 24 hours, and firefly luciferase activity was measured using a Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocol.

Mice

Egln1$^{F/F}$, αMHC-Cre, Cre^{ER}, HSA-Cre-ER^{T2} and ODD-Luc mice used in these studies have been described previously (Minamishima et al., 2008; Moslehi et al., 2010; Safran et al., 2006; Schuler et al., 2005). Except for the ODD-luc mice, which are on a FVB background for imaging purposes, all mice used for these studies were backcrossed to C57BL/6 at least 8 times. *Egln1* gene deletion was achieved by treating age- and sex-matched *Egln1*$^{F/F}$ mice harboring a TAM-regulated Cre with TAM (1 mg) delivered by i.p. injection. For studies in HSA-Cre-ER^{T2} mice, five days of TAM treatment followed by five days to allow adequate recombination and Egln1 protein turnover was used. For studies in Cre^{ER} mice, studies were performed on day 4, with TAM dosed on days 1-3, in order to perform our experiments prior to manifest polycythemia (Minamishima et al., 2008). Genotyping of Egln1 mice was performed by PCR with the following primers: *Egln1* Fwd1 (for null allele); 5'-TCCATCCAGTCTGAGTTTCTTTCC-3', *Egln1* Fwd2 (for Wt and *floxxed* allele); 5'-AGATGACCTCCCCAACTCTGCTAC-3', *Egln1* Rev (common reverse primer); 5'-CAGTGTTCTGCCTCCATTTAT-3'.

***In Vivo* Bioluminescence Imaging**

In vivo bioluminescence imaging in ODD-luc mice was performed as previously described (Safran et al., 2006). Mice were treated with FG-4497 (i.p.) at the stated doses 2 hours prior to administration of D-luciferin (150 mg/kg, i.p.). Bioluminescence images of isoflurane-anesthetized mice were obtained using an IVIS imaging system (Xenogen, Alameda, CA, USA). Representative images are shown.

Western Blot Analysis

Mouse tissue fragments (~ 50 mg) were homogenized in a 500 µl ice cold buffer containing 10 mM Tris-HCl (pH 7.8), 1.5 mM $MgCl_2$ and 10 mM KCl supplemented with protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA), 1 mM Sodium Orthovanadate, 0.5 mM dithiothreitol and 0.4 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich) using an automatic tissue homogenizer (TissueLyser II, Qiagen Inc., Valencia, CA, USA). The homogenates were centrifuged at 4500 *x g* for 5 minute at 4 °C. For some of the experiments, nuclear and cytoplasmic fractions of the homogenized tissue were prepared using a nuclear and cytoplasmic extraction kit (NE-PER, Thermo Scientific,

Waltham, MA, USA). Extracts of cell culture cells were prepared on ice using with lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.5% NP-40) supplemented with protease inhibitor cocktail. Equal amounts of protein extract, as determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA), were resolved by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked with TBST with 5% nonfat dry milk and probed with the following primary antibodies: rabbit polyclonal anti-HIF1α (NB100-479; Novus, Littleton, CO or AG10001; A&G Pharmaceuticals, Columbia, MD, USA), rabbit polyclonal anti-HIF2α (NB100-122; Novus), tubulin (T5468, Sigma), mouse monoclonal anti-vinculin (V9131; Sigma-Aldrich), HIF1α (610959, BD Transduction Laboratories), EGLN1 (4835, Cell Signaling), SNF5 (ab12167, Abcam, Cambridge, MA, USA) or stained with Ponceau (Sigma-Aldrich, St. Louise, MO, USA). Bound antibody was detected with horseradish peroxidase-conjugated secondary antibodies (31430/31432; Pierce, Rockford, IL, USA) and Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA). Protein loading was also standardized using Ponceau S stain.

mRNA Analysis

RNA was purified with TRIzol (Invitrogen, Carlsbad, CA, USA) from cells in culture or homogenized tissues prepared with a TissueLyser II. cDNA was made from 500 ng of total RNA by reverse transcription with random primers (StrataScript First Strand cDNA Synthesis Kit, Stratagene, La Jolla, CA, USA) and analyzed using RT^2 SYBR Green/Rox Master Mix (Qiagen, Valencia, CA, USA) and a Mx3000P thermocycler (Stratagene). Unless otherwise indicated, mRNA levels were normalized to levels of beta-actin mRNA. Forward (F) and reverse (R) PCR primers are as follows:
Egln1 (F,R) 5'-GCCCAGTTTGCTGACATTGAAC-3', 5'-CCCTCACACCTTTCTCACCTGTTAG-3'
Ido1 (F,R) 5'-AGGCTGGCAAAGAATCTCCT-3', 5'-CCAGACCATTCACACACTCG-3'
Ido2 (F,R) 5'-TGAGCATTGCAAGGAAAGTG-3', 5'-TATAGGCCATCAGGCAGTCC-3'
Tdo (F,R) 5'-CCAGCTGCTGACCTCACTTA-3', 5'-CCTTGTACCTGTCGCTCACA-3'
Afmid (F,R) 5'-TTGGGAATTCGTGCAGATAGG-3', 5'-CAGTTTCTCCCCTTCGCCATC-3'
KAT1 (*Ccbl1*; F,R) 5'-CGAAGGCTGGAAGGGATCG-3', 5'-GCGGTGAGAAGTCAGGGAA-3'
KAT2 (*Aadat*; F,R) 5'-ATGAATTACTCACGGTTCCTCAC-3', 5'-AACATGCTCGGGTTTGGAGAT-3'
KAT3 (*Ccbl2*; F,R) 5'-TTCAAAAACGCCAAACGAATCG-3', 5'-GATGACCAAAGCCTCTTGTGT-3'
KAT4 (*Got2*; F,R) 5'-GGACCTCCAGATCCCATCCT-3', 5'-GGTTTTCCGTTATCATCCCGGTA-3'
Sostdc1 (F,R) 5'-CCTGCCATTCATCTCTCTCTCA-3', 5'-CCGGGACAGGTTTAACCACA-3'
Klk4 (F,R) 5'-CCGGATCATACAAGGCCAGG-3', 5'-TGCGGATGCACCAAGACTC-3'
Ctse (F,R) 5'-GACATCAGTCCCTTCGGAAGA-3', 5'-AGGGGTTCATTGACACTCGAATA-3'
Plac9 (F,R) 5'-GTGCAAAGGCGGTTAGACATT-3', 5'-GCCCTGTGGGAAGGTTTGA-3'
Id1 (F,R) 5'-CCTAGCTGTTCGCTGAAGGC-3', 5'-CTCCGACAGACCAAGTACCAC-3'
Mccc2 (F,R) 5'-GCCTATCACGGGGACTCAGT-3', 5'-CACTCCCTCCTAGTCTCACATAC-3'
Lypd1 (F,R) 5'-GGCATCGCAGCAACTTTTTG-3', 5'-GGGATGAGCAATCGTTGTTCAG-3'
Egln3 (F,R) 5'-AGGCAATGGTGGCTTGCTATC-3', 5'-GCGTCCCAATTCTTATTCAGGT-3'
Bnip3 (F,R) 5'-GACGAAGTAGCTCCAAGAGTTCTCA-3', 5'-CTATTTCAGCTCTGTTGGTATCTTGTG-3'
Glut1 (F,R) 5'-TGTCTTGTCACTTTGGCTGGC-3', 5'-AGTATGTGGAGCAACTGTGCGG-3'
Epo (F,R) 5'-TAGCCTCACTTCACTGCTTCG-3', 5'-GCTTGCAGAAAGTATCCACTGT-3'
Vegf-A (F,R) 5'-CCACGTCAGAGAGCAACATCA-3', 5'-TCATTCTCTCTATGTGCTGGCTTT-3'

Ischemia-Reperfusion Injury

Eight to twelve week old mice were anesthetized, intubated and mechanically ventilated. Left thoractotomy was performed, and the left anterior descending (LAD) artery was ligated with a 6.0 silk suture. Ischemia was confirmed by myocardial blanching and electrocardiographic evidence of injury. Five minutes into ischemia, 50 µL of fluorescent microspheres (10 µM FluoSpheres, Molecular Probes, Eugene, OR, USA) was injected into the left ventricular cavity. After 30 minutes, the LAD ligature was released and reperfusion was confirmed visually and electrocardiographically. Sham ischemia-reperfusion experiments were performed in select experiments, where thoracotomy was performed without LAD ligature. Overall survival was 70-80% at 24 hours. Mice were terminally anesthetized 24 hours after ischemia with ketamine / xylazine followed by cervical dislocation, hearts were harvested, and the ventricles were sectioned from apex to base in 2 mm sections. To delineate the infarct size, sections were incubated in 2% (wt/vol) triphenyltetrazolium (TTC, Sigma) in phosphate-buffered saline at 25˚C for 30 minutes. Infarct size and area at risk (AAR) was quantified from light and fluorescent micrographs of

myocardial sections using Adobe Photoshop. Percent myocardial infraction (%MI) was calculated as the total infarction area divided by the total AAR.

Assessment of ischemia-reperfusion injury *ex vivo* in the Langendorff Model was as described previously (Liao et al., 2012). The mice were heparinized and anesthetized. A thoracotomy was performed and heart was rapidly excised. The aorta was cannulated and the heart was perfused retrograde at a constant flow of ~3 ml/min perfusate buffer (124 mM NaCl, 25 mM $NaHCO_3$, 11 mM dextrose, 4 mM KCl, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 2.8 mM $CaCl_2$). A fluid-balloon was inserted into the left ventricle via the mitral valve and connected to a pressure transducer for continuous recording of the left ventricular chamber pressure. Langendorff-perfused hearts were subjected to a stabilization perfusion for 15 minutes followed by global ischemia for 15 minutes and reperfusion for at least 30 minutes. Test compounds (αKG, KynA) stock solutions were prepared at 20x final concentration in perfusate buffer and then combined to the perfusate line via a syringe pump set to $1/20^{th}$ the total flow rate.

Parabiosis Experiments and Assessment of Cross-Circulation

Parabiosis experiments were performed as described previously (Wright et al., 2001). Briefly, mice were anesthetized to full muscle relaxation with ketamine and xylazine by i.p. injection. After shaving the opposing lateral aspects of each mouse to be joined, matching skin incisions were made from the olecranon to the knee joint of each mouse and the subcutaneous fascia was bluntly dissected to create about ½ cm of free skin. The skin was attached by a single 2-0 silk suture and tie and the dorsal and ventral skins were approximated by staples or continuous suture. Ten days after the parabiosis operation, a subset (n=2 parabiosed animals) of mice were used for confirming the presence of cross-circulation of blood by Evan's Blue dye injection. One mouse in each joined pair was injected with 100 µl of Evan's Blue into the retro-orbital venous plexus. Peripheral blood was collected before injection, and 120 minutes after injection.

***In Vivo* Delivery of Stable Isotope Tracers**

For infusion studies, standard sterile surgical technique was used to insert 27-gauge polyurethane catheters into the internal jugular vein and contralateral carotid artery of anesthetized mice. The catheters were externalized and plugged at the time of surgery, and after a 3 day convalescence period, catheter lines were attached to infusion apparatus (Instech Laboratories, Inc., Plymouth Meeting, PA, USA). Conscious, unrestrained mice were infused with 0.19 mg/kg/min U-^{13}C-tryptophan (Cambridge Isotopes Laboratories, Inc., Tewksbury, MA, USA) diluted in normal saline. At 30-minute intervals, 20 µl of blood was sampled from the arterial catheter, transferred to PCR tubes, and centrifuged for 10 seconds at 10,000 x *g*. A 10 µl aliquot of plasma was snap-frozen in liquid N_2 and stored at -80°C. After 90 minutes of infusion, a 50 mg/kg bolus of FG-4497 was administered over 4 minutes. 60 minutes later, mice were sacrificed by sodium pentobarbital (120 mg/kg), and tissues were clamped in liquid N_2-cooled forceps, and stored at -80°C until analyzed by mass spectrometry.

Metabolite Extraction and Mass Spectrometry

To harvest mouse tissues for metabolic analyses, organs were quickly dissected from terminally anesthetized animals, and a small (~50 mg) tissue section was clamped between liquid nitrogen-cooled flat forceps, weighed accurately, and ground using a TissueLyser II that had been cooled in liquid nitrogen. Extraction of metabolites from ground tissue was performed by vortexing ground tissue in cold methanol:water:chloroform (6:3:4) for 10 minutes at 4°C followed by centrifugation at 10,000 x *g* for 10 minutes at 4°C to separate fractions. For selected experiments, the extraction water contained 2 mg/ml norvaline (Nor, Sigma-Aldrich, St. Louis, MO, USA) as an internal standard for normalization. Aliquots of aqueous fractions, in proportion to tissue exact mass, were transferred to autosampler vials and dried at 4°C in a CentriVap (Labconco, Kansas City, MO, USA) in duplicate. For cell culture experiments, sample extraction were carried out in methanol:water:chloroform according to published methods (Fendt et al., 2013).

For gas chromatography mass spectrometry (GCMS) analyses, dried metabolites were derivatized in 2% methoxyamine hydrochloride in pyridine (MOX, Sigma) followed by N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide + 1% tert-butyldimethylchlorosilane (TBDMS, Sigma) according to published methods (Fendt et al., 2013). One microliter of derivatized sample was injected into an Agilent 7890B GC with a 30m DB-35MS capillary column in line with an Agilent 5977T MS. A splitless inlet liner and inlet temperature of 270°C was used with helium carrier gas flow rate of 1 mL/min. Electron impact

ionization energy was 70eV, ion source temperature was 120°C, and quadrupole temperature was 150°C. Ion detection was performed in Scan Mode (m/z between 100 and 605). Cell culture data were normalized to internal standard norvaline and cell number. For analysis of tissue metabolites, ion counts were normalized to the area under the entire chromatogram. Reported are the dominant ions for derivatives alpha-ketoglutarate (αKG, m/z 346), Succinate (Suc, m/z 289), and Glutamate (Glu, m/z 432). ^{13}C mass isotopes were quantified and corrected for naturally occurring mass isotope abundances using an in-house software(Antoniewicz et al., 2007) run in Matlab v2009b (Mathworks).

For liquid chromatography mass spectrometry LC-MS analysis of tissue metabolites, acetonitrile:methanol:formic acid (75:25:0.5 v:v:v) containing isotope-labeled internal standards was used to resuspend dried polar metabolites or directly extract metabolites from serum and plasma samples. Samples were analyzed in positive and negative ion mode using via hydrophilic interaction liquid chromatography (HILIC) MS analyses and previously described protocols (Avanesov et al., 2014; Townsend et al., 2013; Wang et al., 2011). Samples were analyzed using a Nexera X2 U-HPLC (Shimadzu, Marlborough, MA) and a Q-Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific; Waltham, MA), and data were processed using Tracefinder (version 3.2, Thermo Fisher Scientific; Waltham, MA).

Invasive Hemodynamic Monitoring

Mice were anesthetized with isofluorane, intubated, and ventilated. After sterile dissection of the carotid artery, a Millar Mikro-Tip pressure catheter (Millar, Houston, TX, USA) was inserted trans-carotid into the aortic arch. Pressures were recorded using a Millar MPVS-300 controller and LabChart software (ADInstrument, Colorado Springs, CO, USA).

Nitrite Measurement

Mice were anesthetized with isoflurane and 50-100 μl of blood was harvested from the retro-orbital venous plexus using a capillary tube that was pre-washed with nitrite-free water. Blood was immediately transferred directly into 400 μl of Nitrite-Preservation Solution (0.8 M potassium ferricyanide, 0.1 M N-ethylmaleimide, 10% Nonidet P-40, Sigma), carefully weighed, and frozen at -80°C in gas impermeable microfuge tubes. Nitrite was quantified by ozone-based chemiluminescence in a Nitric Oxide Analyzer (Sievers 280i, GE Analytical Instruments, Boulder CO, USA) according to published protocols (Pelletier et al., 2006).

Microarray and Gene Set Enrichment Analysis

Tissue RNA was hybridized to Affymetrix Mouse Gene 1.0 ST arrays processed by the Dana Farber Core Facility. All data processing was performed with the R programming language. CEL files were RMA normalized with the *affy* R package. Differential gene expression was determined using the Empiric Bayes library in the R *limma* package. Correction for multiple hypotheses was determined using the Benjamini-Hochberg method. Gene set enrichment analysis was performed with the javaGSEA software available from the Broad institute. Secreted proteins were selected from a published dataset (Wu et al., 2010).

Cytokine Array and ELISA

Egln1$^{F/F}$;HSA-Cre-ER^{T2} and *Egln1*$^{+/+}$;HSA-Cre-ER^{T2} mice (n=3 each) were treated with TAM as described in Figure 4. WT mice treated with IFNγ (10 or 30 μg, 24 hours) were included as a positive control (data not shown). Blood was collected from the retro-orbital venous plexus of isoflurane-anesthetized mice, and serum was frozen at -80°C. An aliquot of frozen serum was used for cytokine array (CYT-G2000, RayBiotech Inc., Norcross, GA, USA) according to the manufacturer's protocol. Fold change and statistical significant (Student's t-test) was calculated by dividing median-normalized data from *Egln1*$^{F/F}$;HSA-Cre-ER^{T2} mice by median-normalized data from *Egln1*$^{+/+}$;HSA-Cre-ER^{T2}. IL-10 (Fisher Scientific, Pittsburgh, PA, USA) and Epo (R&D Systems, Minneapolis, MN, USA) ELISAs were performed on aliquots of serum according to manufacturer's protocol.

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